Development 110, 1197–1207 (1990)
Printed in Great Britain © The Company of Biologists Limited 1990

Expression and regulation of the *abd-A* gene of *Drosophila*

ANA MACIAS, JORDI CASANOVA and GINES MORATA

Centro de Biología Molecular (CSIC-UAM), Universidad Autónoma, Canto Blanco, 28049 Madrid, Spain

Summary

We have developed a specific polyclonal antibody that recognizes the protein products of the *abdominal-A* (*abd-A*) gene, a member of the bithorax complex of *Drosophila*. The normal expression domain extends from parasegments 7 to 13, in good correspondence with previous genetic and molecular results. However, while the anterior border of expression is precisely demarcated by a parasegmental boundary, the posterior border does not coincide with a lineage boundary. Within the normal domain, the expression of *abd-A* shows intrametameric modulation; the amount of product is higher in posterior compartments and in the most anterior cells of the anterior compartments and then gradually decreases. We have examined the effect on *abd-A* expression of a number of mutations, some mapping within and others outside the *abd-A* transcription unit. Those mapping to the transcription unit eliminate or severely reduce the amount of *abd-A* antigen, while those mapping outside produce an abnormal distribution of *abd-A* protein. Finally, we show that the *abd-A* gene is down-regulated in part of the *Abdominal-B* (*Abd-B*) domain, precisely in those regions where the *Abd-B* gene is expressed at high levels.

Key words: bithorax complex, parasegments, *abd-A* antibody.

Introduction

The homeotic genes of *Drosophila*, which are clustered in the Antennapedia (ANT-C) and bithorax (BX-C) complexes (Lewis, 1978; Sánchez-Herrero *et al.* 1985; Mahaffey and Kaufman, 1988), establish the characteristic development (identity) of the body segments or parasegments (Martínez-Arias and Lawrence, 1985). The BX-C is composed of three genes, *Ultrabithorax* (*Ubx*), *abdominal-A* (*abd-A*) and *Abdominal-B* (*Abd-B*), and specifies the identities of parasegments 5 to 14 (Sánchez-Herrero *et al.* 1985; Casanova *et al.* 1987), which include part of the thorax and the entire abdomen. The gene *abd-A* is necessary for the normal identities of most abdominal segments.

One fundamental feature of the homeotic genes is that their areas of activity are defined by compartment boundaries (see Morata and Lawrence, 1977). This correlation between domains of activity and of cell lineage is of importance for it suggests that homeotic gene activities, which perdure for most of the development (Morata and Garcia-Bellido, 1976), are maintained by cell lineage. In several well-studied cases, *Ubx*, *Antennapedia* (*Antp*), *Deformed* and *Sex combs reduced*, (Akam and Martinez-Arias, 1985; White and Wilcox, 1985; Wirz *et al.* 1986; Jack *et al.* 1988; Mahaffey and Kaufman, 1987; LeMotte *et al.* 1989), the anterior border of expression coincides with an antero-posterior compartment boundary. The posterior borders are not so well defined and, in *Ubx* and *Antp* (Struhl and White, 1985; Wirz *et al.* 1986), they do not correspond to a known compartment line. Furthermore, the levels of expression in the posterior part of the domain seem to depend on interactions with other homeotics (Hafen *et al.* 1984; Struhl and White, 1985; Wirz *et al.* 1986).

Within their own domains, the homeotic genes present local variations of expression. *Ubx*, for example, is less expressed in parasegment 5 than in 6 (Akam and Martinez-Arias, 1985; White and Wilcox, 1985) and within the individual metameres, the *Ubx* product is less abundant in the posterior compartments (Martinez-Arias and White, 1988). In the *Abd-B* domain, there are quantitative and also temporal differences in different metameres (Kuziora and McGinnis, 1988; Sánchez-Herrero and Crosby, 1988; Celniker *et al.* 1989; DeLorenzi and Bienz, 1990).

It is clear that there must be a number of factors responsible for these complex spatial and temporal regulations. *Trans* acting products like those of *Polycomb* (Lewis, 1978) or of segmentation genes (White and Lehmann, 1986; Martinez-Arias and White, 1988) as well as regulatory elements of the homeotic genes themselves (Casanova *et al.* 1985, 1987; Peifer and Bender, 1986; DeLorenzi and Bienz, 1990) are involved in the process.

The expression of *abd-A* is not well characterized. Genetic results (Sánchez-Herrero *et al.* 1985; Casanova *et al.* 1987), *in situ* hybridization to tissue sections (Harding *et al.* 1985; Rowe and Akam, 1988) and the expression of the gene in the visceral mesoderm (Tremml and Bienz, 1989) indicate that the limits of its

expression are defined by parasegmental borders, but a precise definition of its embryonic domain is still needed.

The genetic and molecular structure of *abd-A* is known is some detail (Karch *et al.* 1985; Busturia *et al.* 1989). It comprises around 60 kb of DNA and appears to have just one homeobox-containing transcription unit (Karch *et al.* 1985; F. Karch, personal communication). Some mutations map within and others outside the transcription unit, suggesting that they affect distinct functions of the gene. From genetic studies (Busturia *et al.* 1989), there is evidence of the existence of at least two regulatory regions, one 5′, the other 3′ to the transcription unit.

Further characterization of the different regulatory regions of *abd-A* is of importance in connection with the problem of the spatial regulation of the expression of the BX-C genes. Current models (Lewis, 1978; Peifer *et al.* 1987; Casanova *et al.* 1987) propose the existence of parasegment-specific regulatory elements similar to those found for the *Ubx* gene (Casanova *et al.* 1985; White and Wilcox, 1985; Beachy *et al.* 1985; Peifer and Bender, 1986), but presently there is little evidence for their existence in the case of *abd-A* (Busturia *et al.* 1989) and *Abd-B*.

By using a specific anti-*abd-A* antibody, we address some of the problems about the expression and regulation of *abd-A*. We describe the embryonic domain of *abd-A* expression and how it is affected by different classes of *abd-A* and *Abd-B* mutations.

Materials and methods

(1) *Mutant stocks and crosses*

All mutations in the *abd-A* and *Abd-B* genes used in this work have been described in Sánchez-Herrero *et al.* 1985; Karch *et al.* 1985; Casanova *et al.* 1986 and Busturia *et al.* 1989.

To facilitate the identification of mutant embryos for *abd-A*, we have used a *TM3 hb-β-gal* balancer chromosome recently made by Dr Gary Struhl. This offers the advantage that any homozygous mutant embryo in a stock balanced with *TM3 hb-β-gal* can be readily recognized after double staining with *β-gal* and *abd-A* antibodies because it does not contain *β-gal* antigen in the *hb* domain. Further details on this chromsome are to be provided by Dr Struhl (in prep.).

(2) *Production of the abd-A* antibody

The *pabexl* clone transformed into K38 cells was obtained from Jeff Simon (Harvard Medical School). This clone contains a 1.1 kb *Nhe*I–*Sma*I fragment from an *abd-A* cDNA inserted into pT7-7. A protein from this cDNA was overproduced as described in Tabor and Richardson, 1985. After induction, cells were centrifuged, washed and resuspended in 10% sucrose 20 mM Tris pH 8 and 1 mM EDTA. After incubation for 45 min with lysozyme, the suspension was frozen in liquid nitrogen and thawed twice, sonicated and, after adding NaCl to 0.5 M, it was centrifuged for 30 min at 10 000 rev min^{-1}. The pellet was used to immunize two rats. After two boosts, an antiserum was obtained and tested for immunostaining. As described in the main text, this antiserum specifically recognizes *abd-A* protein.

(3) *Antibody staining*

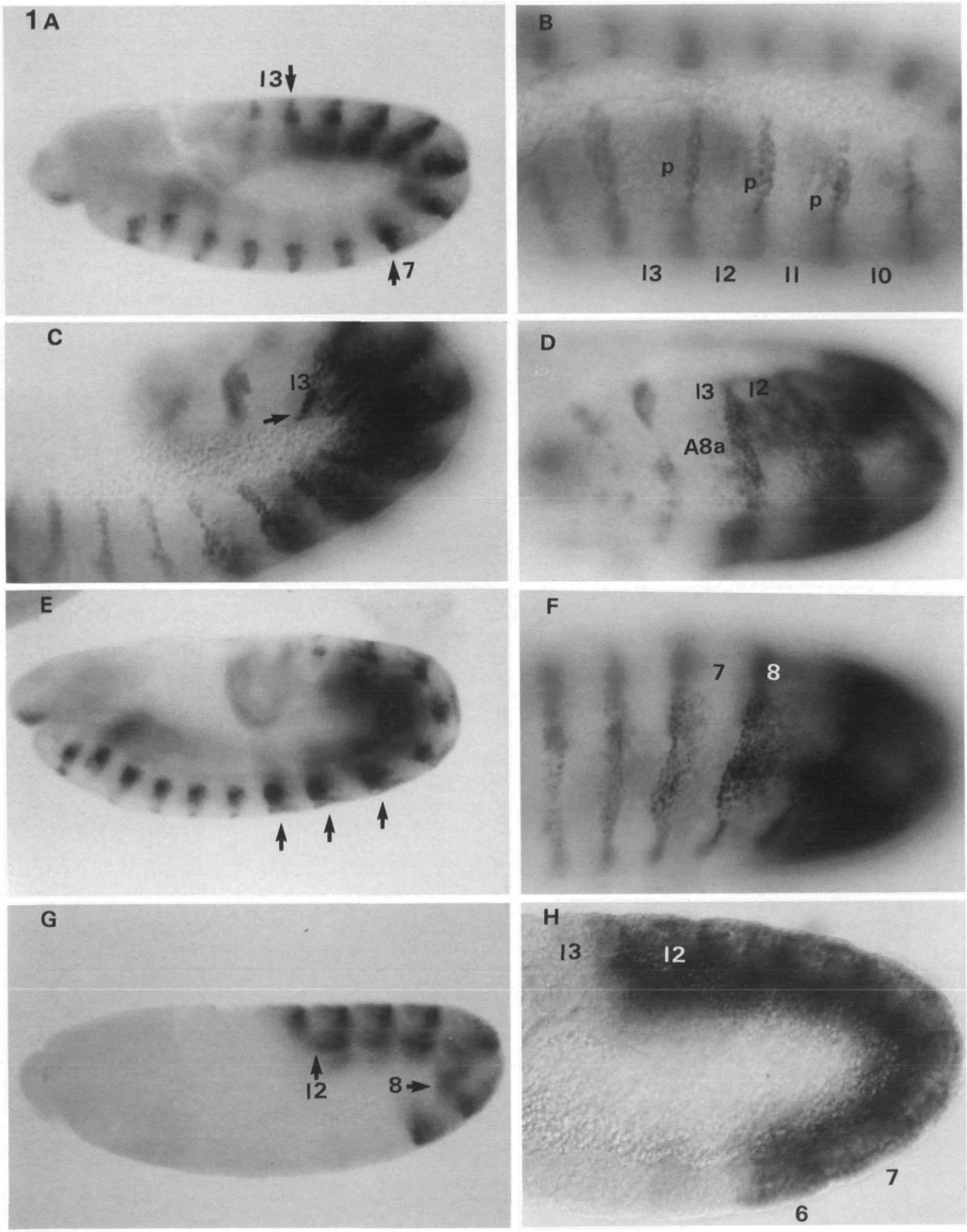
For immunodetection, embryos were dechorionated in bleach, washed and fixed in PEM (Pipes 0.1 M pH 6.9; 1 mM EGTA; 2 mM $MgSO_4$), 4% formaldehyde/heptane 1:3 for 20 min with continuous shaking. Then, the vitelline membrane was removed in heptane/methanol and the embryos collected and stored in methanol. For labelling, the embryos were hydrated and permeabilized in BBT (BBS pH 7+0.1% BSA+0.1% Triton) for 30 min and treated with the primary antibody overnight at 4°C. After several washings in BNT (BBT+2% goat serum), they were incubated with biotinylated secondary antibody (Amersham) at a dilution 1:300 for 2 h at room temperature and washed in PBT (PBS+0.05% Tween 20). The embryos were reacted with the Vectastain ABC Kit (Vectorlabs) and stained with DAB (0.5 mg ml^{-1})/PBT plus 2 μl of 3% H_2O_2. In the case of double-labelling experiments (Lawrence *et al.* 1987), the same process was done twice, with the difference that the second staining included 0.02% nickel sulphate and 0.02% cobalt chloride. The embryos were dehydrated in alcohol series, embedded in Araldite/acetone and mounted in Araldite. The working dilutions for the primary antibodies were anti-*abd-A* 1:500, anti-*invected* 1:500, anti-*β-gal* (Cappel) 1:2000 and anti-*Ubx* 1:1. The anti-*invected* antibody was obtained from Dr T. Kornberg and the anti-*Ubx* from Dr R. White.

Results

(1) *Expression of the* abd-A *protein in wild-type embryos*

For the analysis of the expression of *abd-A* protein in different embryonic periods, we have produced a polyclonal rat anti-*abd-A* antibody (see Materials and methods). The specificity of the antibody is revealed by the absence of staining in the abdominal region of embryos homozygous for deficiencies of *abd-A*. However, in those embryos after germ band retraction, the

Fig. 1. Expression of *abd-A* protein at the extended germ band period. Embryos of pictures A–F are doubly stained for *engrailed* (blue) and *abd-A* (ochre) (A) Stage 9–10 embryo showing the general domain of *abd-A* expression from parasegments 7 to 13 (arrows). ×200. (B) Lateral view of parasegments 10–13 in an embryo of stage 10. The expression of *abd-A* is restricted to posterior compartments and to cells around the tracheal pits (p). Note that there is no label in the tracheal pit of A8a. ×400. (C) Lateral view of an embryo in the process of germ band retraction. The posterior border of *abd-A* antigen coincides with that of *en* (arrow), indicating that unlike the anterior one, it is a segmental boundary. (D) Ventral view of parasegments 11, 12 and 13 of an embryo of the same age as the previous one. Some, but not all, the cells of A8a are labelled for *abd-A*. The pattern of expression in 13 is clearly different from 11 and 12. (E) Embryo retracting the germ band showing in the epidermal cells (arrows) the gradient of parasegmental expression. (F) Differential expression of *abd-A* antibody in parasegments 7 and 8. There is less amount of product in 7 than in 8. (G) Simple staining for *abd-A* to show the differences of *abd-A* expression in the ectodermal and mesodermal layers. The staining in the mesoderm extends from parasegment 8 to 12 (arrows). (H) Embryo doubly stained for *Ubx* (ochre) and *abd-A* (blue) to show the reciprocal levels of expression of these two genes. Some parasegments are indicated.


1A
I3
7
B
p
p
p
I3
I2
I1
I0
C
I3
D
I3
I2
A8a
E
F
7
8
G
I2
8
H
I3
I2
7
6

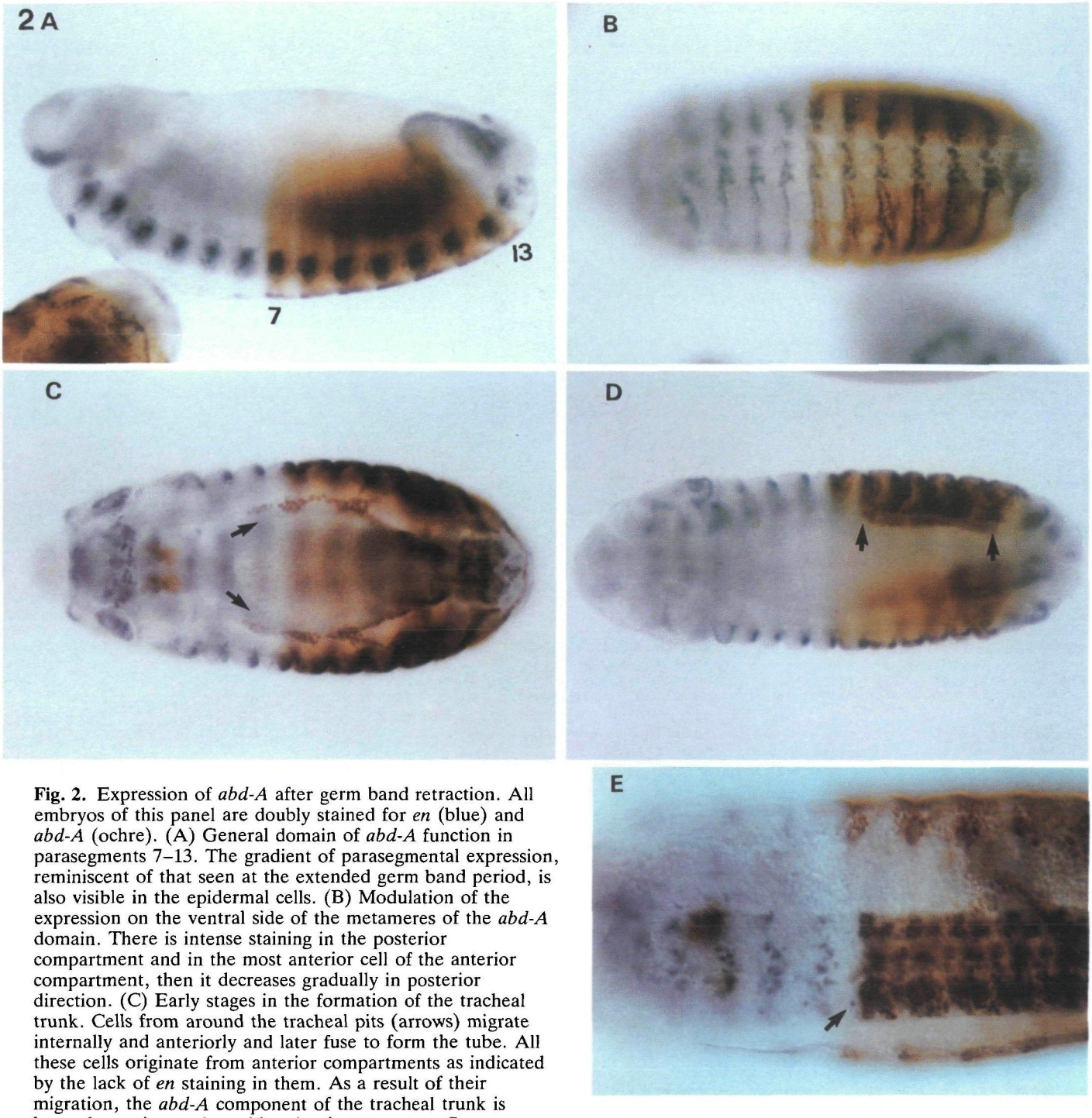


Fig. 2. Expression of *abd-A* after germ band retraction. All embryos of this panel are doubly stained for *en* (blue) and *abd-A* (ochre). (A) General domain of *abd-A* function in parasegments 7–13. The gradient of parasegmental expression, reminiscent of that seen at the extended germ band period, is also visible in the epidermal cells. (B) Modulation of the expression on the ventral side of the metameres of the *abd-A* domain. There is intense staining in the posterior compartment and in the most anterior cell of the anterior compartment, then it decreases gradually in posterior direction. (C) Early stages in the formation of the tracheal trunk. Cells from around the tracheal pits (arrows) migrate internally and anteriorly and later fuse to form the tube. All these cells originate from anterior compartments as indicated by the lack of *en* staining in them. As a result of their migration, the *abd-A* component of the tracheal trunk is located anterior to the epidermis of parasegment 7. (D) Expression in the visceral mesoderm from parasegment 8 to 12 (indicated by arrows). Note the lack of *engrailed* label. (E) Expression in the embryonic central nervous system. There is not a complete coincidence in the expressions of *en* and *abd-A*; notice the *en*-stained neuron (arrow), which is not labelled by *abd-A*.

antibody marks some cells near the brain. Although this label is clearly unrelated to *abd-A*, it is a useful internal control of the staining procedure in the case of *abd-A* mutants.

Precise limits of expression were determined by double-labelling experiments (Lawrence *et al.* 1987) with anti-*abd-A* and either anti-*engrailed* (Patel *et al.* 1989) or anti-*Ubx* (White and Wilcox, 1984) antibodies. This permits us to locate the *abd-A* domain with respect to those of *en* and *Ubx*.

The first sign of *abd-A* protein appears during the stages 9–10 of development (Campos-Ortega and Hartenstein, 1985), at the extended germ band period (Fig. 1A). The label is nuclear and appears simultaneously in parasegments 7–13, precisely the parasegments where the lack of function *abd-A* mutations produce a phenotype (Sánchez-Herrero *et al.* 1985; Busturia *et al.* 1989). Cells in the posterior compartments and those around the tracheal pits are the most intensely stained (Fig. 1B). The anterior border of *abd-A* expression appears to coincide with parasegment 7, for, as far as we can judge, the coincidence of *en* and *abd-A* expression in A1p is complete (Fig. 1A,C). Thus, the anterior limit of *abd-A* expression is strictly parasegmental. Not so the posterior limit, which is segmental laterally and does not define a compartment border on the ventral side (Fig. 1C,D); some ventral cells in A8a are the most posteriorly stained cells with *abd-A*. Since the *abd-A*$^-$ mutations transform the denticle belt in A8a (Sánchez-Herrero *et al.* 1985), we presume that these cells include the precursors of the A8a larval epidermis. The mesoderm layer also contains *abd-A* protein, which is out of register with respect to the ectoderm by approximately one parasegment; it extends from parasegment 8 to 12 (Fig. 1G).

The expression of *abd-A* appears to be modulated in a metameric fashion. This can be observed in embryos of stage 11, when *abd-A* expression is well established; the cells of the posterior compartments are strongly labelled and in the anterior compartment there is a gradient of intensity diminishing posteriorward (Fig. 1E,F). The cells just anterior to the *en* stripe have almost no label. With the exception of parasegment 13, this pattern is reiterated in every metamere, although parasegment 7 (and possibly 8) contains less *abd-A* protein than the rest. This modulation appears to be complementary to that described for *Ubx* (Struhl and White, 1985; Martinez-Arias and White, 1988). In Fig. 1H we illustrate this by a double staining for *Ubx* and *abd-A*; the regions with a high level of *abd-A* antigen show little *Ubx* and *vice versa*.

After germ band retraction (Fig. 2A), the segmental grooves are visible and *abd-A* protein is observed on both sides of the grooves, from A1/A2 to A7/A8; the expression in A1p coincides with that of *engrailed*. In A8, however, the *abd-A* label only appears in some cells in the anterior compartment, as in earlier stages. In the intervening segments, we observe a repeated pattern of expression reminiscent of that seen at the extended germ band period; the strongest label appears in the posterior compartment cells and in the most anterior cells of the anterior compartment. Then it decreases gradually in the posterior direction, although there is always label above background (Fig. 2B). It is of interest that the amount of *abd-A* protein in A4, A5, A6 and A7 is the same or slightly higher than in A2 and A3, even though in A5, A6 and A7 there is also *Abd-B* protein (Celniker *et al.* 1989; DeLorenzi and Bienz, 1990). This suggests that there is no down-regulation of *abd-A* by *Abd-B* in these segments.

After germ band retraction, cells from around the tracheal pits migrate internally and anteriorly to form the tracheal tree (see Campos-Ortega and Hartenstein, 1985). These cells stain for *abd-A* but not for *en* indicating that they all originate from anterior compartments. As a consequence of their migration, the anterior limit of *abd-A* expression in the tracheal tree has moved anteriorly with respect to the epidermis (Fig. 2C). By stage 14 the ventral cord matures and shows intense staining for *abd-A*. From the double staining with *en*, it can be seen that the anterior limit of *abd-A* in the central nervous system (CNS) corresponds with the border of parasegment 7. However, we observe that the coincidence of the *en* and *abd-A* labels is not always cell by cell; there are two neurons (Fig. 2E) on each side, marked by *en*, that do not contain detectable levels of *abd-A*.

The expression in the visceral mesoderm can be clearly determined after germ band retraction (Fig. 2D). It follows the same rule described for the mesoderm layer; its anterior limit is offset one parasegment with respect to the epidermis, starting at parasegment 8, as has been noted before (Tremml and Bienz, 1989), and ending at parasegment 12. We find no *en* label in the mesoderm indicating that in this layer there is no distinction between anterior and posterior compartments, in agreement with previous observations (Lawrence, 1982).

After the germ band shortening, the staining of the amnioserosa cells can be readily studied. As these cells are not organized in segments, it is not possible to ascertain their segmental origin. We find *abd-A* protein in amnioserosa cells from the level corresponding to A3–A4 segments in the epidermis down to the end of the abdomen, although the most posterior cells are less strongly labelled.

(2) *Different classes of* abd-A *mutations affect* abd-A *expression differently*

In many of the experiments aimed to study the expression of the gene product in *abd-A* mutations, we have used stocks with a balancer chromosome carrying a *β-gal* insert (see Materials and methods). Thus embryos homozygous for the mutation can be recognized for the lack of anti-*β-gal* staining.

We have studied three types of *abd-A* mutations: (1) deletions of the gene like *Df109* and *DfP9*; (2) mutations mapping within the *abd-A* transcription unit: the alleles *M1*, *MX1*, *MX2*, *C26*, *P10* and *C1* and (3) mutations mapping outside, either 5′ (*iab-3*^{277}, *iab-3*^{MX47}, *iab-3*^{Uab4}, *iab-4*^{MX4} and *iab-4*^{302}) or 3′ (*iab-2*^{k}, *iab-2*^{M1} and *iab-2*^{Uab1}) to the transcription unit.

Embryos homozygous for *Df109* of *DfP9* only show staining in the cells near the brain and nowhere else. Embryos carrying mutations in the transcription unit produce a reduction or elimination of *abd-A* product. The alleles *M1* and *MX1* show no *abd-A* antigen (Fig. 3C), whereas in *MX2* and *P10* there is some, the amount of product in *P10* being greater. In the case of *P10*, the diminution is not uniform; there is a gradient of product from almost none in A2 to higher levels in A6 and A7 segments (Fig. 3B). These observations fit

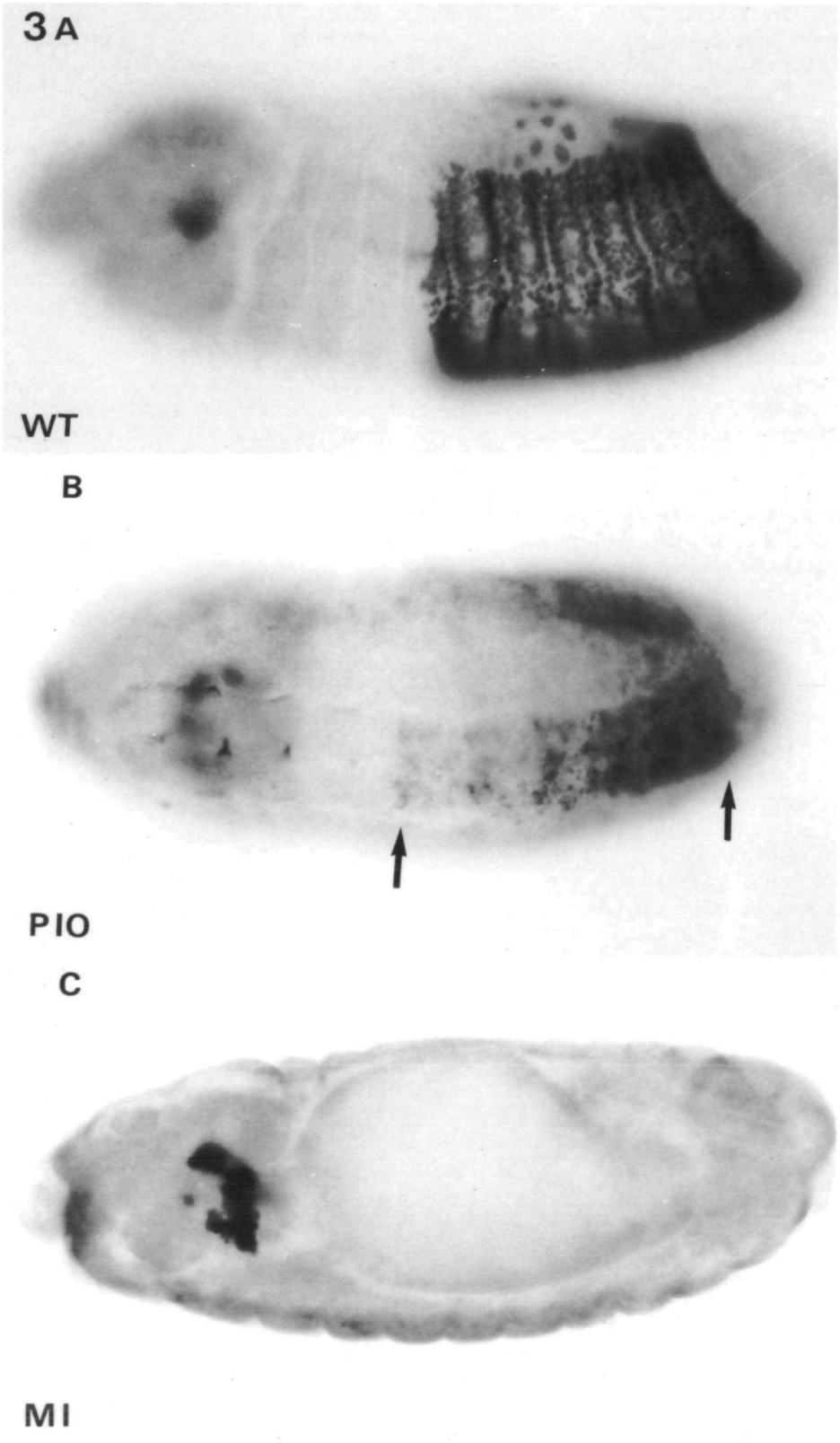


Fig. 3. *abd-A* expression in (A) wild-type, (B) $abd\text{-}A^{P10}$ and (C) $abd\text{-}A^{M1}$ embryos. In B, there is a gradient in the amount of antigen, which is particularly clear in the nerve cord, from low in parasegment 7 (arrow) to much higher in 12 (arrow). In C, due to the null nature of the mutation, there is no *abd-A* protein in the domain, only the cells near the brain are labelled.

very precisely with genetic results (Morata *et al.* 1983; Busturia *et al.* 1989) that showed that there is some *abd-A* function in *MX2* and *P10*. The latter is particularly illuminating because it was shown (Morata *et al.* 1983) that *P10* has a segment-dependent graded phenotype, in accordance with the expression of *abd-A* protein. The mutations *MX2* and *P10* are breakpoints mapping to the extremes of the transcription unit (Karch *et al.* 1985), suggesting that they do not affect the coding region but remove some regulatory sequences close to it (see Discussion).

The mutation *C1* is special in that it is a deletion with breakpoints in introns of *Ubx* and *abd-A* producing a hybrid *abd-A–Ubx* product (Rowe and Akam, 1988; Casanova *et al.* 1988) in which *Ubx* provides the carboxyl terminus of the protein and the homeobox. Because the hybrid product is now in part under the control of regulatory elements of *Ubx* (Rowe and Akam, 1988; Casanova *et al.* 1988), the *abd-A* portion of the protein should be expressed in the *Ubx* domain. However, we find that our antibody does not react with the *C1* product, unlike the other extant anti-*abd-A* antibody (F. Karch, personal communication). Our antibody only recognizes epitopes in the carboxyl portion of the protein.

Mutations mapping outside the transcription unit cause a misregulation of *abd-A* function (Busturia *et al.* 1989). Our results support this view; all the mutations tested with the anti-*abd-A* antibody show presence of *abd-A* protein; however, the pattern of expression is abnormal in some of them.

The *iab-2* mutations studied, $iab\text{-}2^{k}$, $iab\text{-}2^{M1}$ and $iab\text{-}2^{Uab1}$, may produce a partial loss of *abd-A* product, but this is hard to ascertain. In $iab\text{-}3^{277}$ and $iab\text{-}3^{MX47}$, we also observe *abd-A* staining in the normal domain, but the amount of antigen in A3–A7 appears slightly reduced with respect to the wild-type. In the two *iab-4* mutations, $iab\text{-}4^{MX4}$ and $iab\text{-}4^{302}$, we do not find any alteration in *abd-A* expression. Unfortunately, the intensity of the *abd-A* stain within the A3–A7 region is quite uniform, so it is not possible to establish whether a given mutation changes, say, the A4 pattern into A3. For this reason, metamere-specific transformations may have been overlooked.

In addition to the effect in the normal domain, the mutations of the *infraabdominal* type frequently produce ectopic expression of the *abd-A* protein, in accordance with genetic data (Busturia *et al.* 1989). We have observed this in $iab\text{-}2^{k}$, $iab\text{-}2^{Uab1}$, $iab\text{-}3^{277}$, $iab\text{-}3^{Uab4}$ and the combinations like $iab\text{-}2^{S3}/iab\text{-}3^{277}$. In general, this is observed in embryos after the germ band has retracted, but in the cases of $iab\text{-}2^{Uab1}$ and $iab\text{-}3^{Uab4}$ it occurs during germ band elongation (Fig. 4C). In $iab\text{-}2^{k}$ the ectopic expression is more marked and it affects the dorsolateral region of the thoracic segments (Fig. 4A) but it is observed only if the embryos are also homozygous for the $su(Hw)^{2}$. This mutation is known to suppress virtually all the *gypsy* induced mutant alleles (Modolell *et al.* 1983). This suggests that the relative loss of function of $iab\text{-}2^{k}$ in the presence of the wild-type allele of *su(Hw)* is due to the presence of the *su(Hw)* product interfering with transcriptional control elements of *abd-A*, (Parkhust and Corces, 1986) and that the genuine effect of the breakpoint of $iab\text{-}2^{k}$ is an ectopic expression of *abd-A* product (Busturia *et al.* 1989).

(3) *Interactions of* abd-A *with* Ubx *and* Abd-B

As we show above, there are reciprocal amounts of *Ubx* and *abd-A* antigens in the metameres of the *abd-A* domain. As *Ubx* is down-regulated by *abd-A* (Struhl and White, 1985), these results suggested that the metameric expression of *Ubx* may be the result of, or influenced by, the levels of *abd-A*. We tested this idea by looking at the expression of *Ubx* in the absence of *abd-A* function. In $abd\text{-}A^{-}$ embryos, most of the *Ubx* modulation is lost (Fig. 5B); the posterior compartments show high levels of *Ubx* activity. As there is normal expression of *en* in $abd\text{-}A^{-}$ embryos, this experiment suggests that, in the *abd-A* domain, *en* does not directly repress *Ubx*; the low level of *Ubx* in posterior compartments in the wild type is due to down-regulation by *abd-A*. However, not all the *Ubx* modulations disappear in $abd\text{-}A^{-}$ embryos; within the anterior compartments there are variations in the amount of product, which probably reflect a dependence of *Ubx* and *abd-A* genes on other upstream genes.

The study of the interactions of *abd-A* with *Abd-B* was particularly intriguing because, unlike other cases of interactions, for examples those of *Antp* and *Ubx*, and of *Ubx* and *abd-A*, (Hafen *et al.* 1984; Struhl and White, 1985), there is no indication of down-regulation of *abd-A* by *Abd-B*, at least as far posterior as parasegment 12. As we point out above, the level of *abd-A* protein in segments A5, A6 and A7 (part of the *Abd-B* domain) is, if anything, higher than in A2 and A3 (part of the *abd-A* domain). Only in A8, corresponding to the high levels of *Abd-B* protein (Celniker *et al.* 1989; De Lorenzi and Bienz, 1990), is there a marked reduction of protein with respect to more anterior segments.

In embryos totally deficient for *Abd-B* functions like those homozygous for $Abd\text{-}B^{M1}$ (Casanova *et al.* 1986), there is a clear alteration of the pattern of *abd-A* expression. There is ectopic presence of *abd-A* protein in parasegments 13, 14 and part of 15, precisely those that in the wild type contain high levels of *Abd-B* product (Kuziora and McGinnis, 1988; Sánchez-Herrero and Crosby, 1988; Celniker *et al.* 1989; DeLorenzi and Bienz, 1990). This derepression, however, does not occur simultaneously; at the extended germ band stage the ectopic expression of *abd-A* affects only cells of parasegment 13, stopping just short of A8p (Fig. 6B). After the germ band has retracted, it extends to parasegments 14 and 15 (Fig. 6C). These results suggest the existence of different mechanisms of control of *abd-A* expression in the posterior regions (see Discussion). They also indicate that the down-regulation of *abd-A* by *Abd-B* requires high levels of *Abd-B* product; the lower amounts of *Abd-B* protein in parasegments 10, 11 and

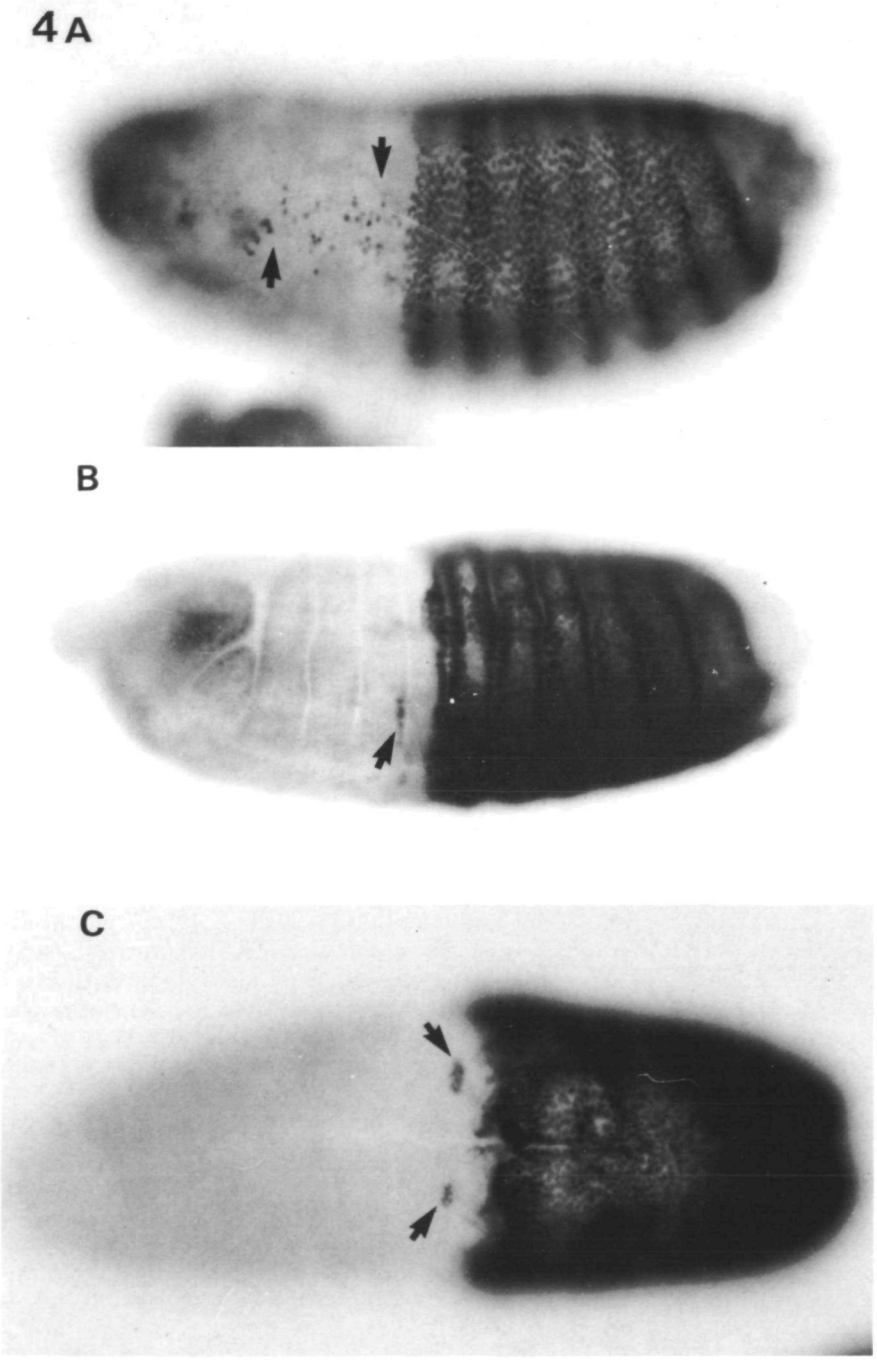


Fig. 4. Ectopic expression of *abd-A* protein in *infraabdominal* mutations. (A) Stage 11 *iab-2*^{k} homozygous embryo also defective in *su(Hw)* function. Note the scattered cells anterior to the normal *abd-A* domain showing presence of the *abd-A* antigen (arrows). (B) Embryo of similar age of genotype *iab-3*^{Uab1} showing cells of parasegment 6 with *abd-A* product (arrow). (C) *iab-3*^{Uab4} embryo at the extended germ band period expressing *abd-A* protein in parasegment 6 (arrows).

12 (Celniker *et al.* 1989; DeLorenzi and Bienz, 1990) having no detectable effect on *abd-A* expression.

Genetic and molecular studies have demonstrated that *Abd-B* contains two distinct functions, *m* and *r*, which are mediated by two different proteins sharing the carboxyl terminus, (Casanova *et al.* 1986; DeLorenzi *et al.* 1988; Sánchez-Herrero and Crosby, 1988; Kuziora and McGinnis, 1988). The *m* function is specific to parasegments 10, 11, 12 and 13, while the *r* function is restricted to 14 and 15.

We have tested the effect on the down-regulation of *abd-A* of both the *m* and the *r* products. In the mutation *Abd-B*^{M5} ($m^{-}r^{+}$), the expression of *abd-A* is high in the whole of parasegment 13, including the region of A8a that in the wild type does not contain *abd-A* protein, and that coincides with the area of high expression of the *m* product. This effect is observed from the extended germ band stage. There is no expression of *abd-A* in parasegments 14 or 15.

In the mutants *Abd-B* $r^{x23\text{-}1}$ and *Abd-B*$^{tuh\text{-}3}$, both $m^{+}r^{-}$, there is adventitious *abd-A* expression in A8p, A9a and sometimes in A9p (parasegments 14 and 15, Fig. 7), but this is only observed after the germ band has retracted. This area of ectopic *abd-A* expression

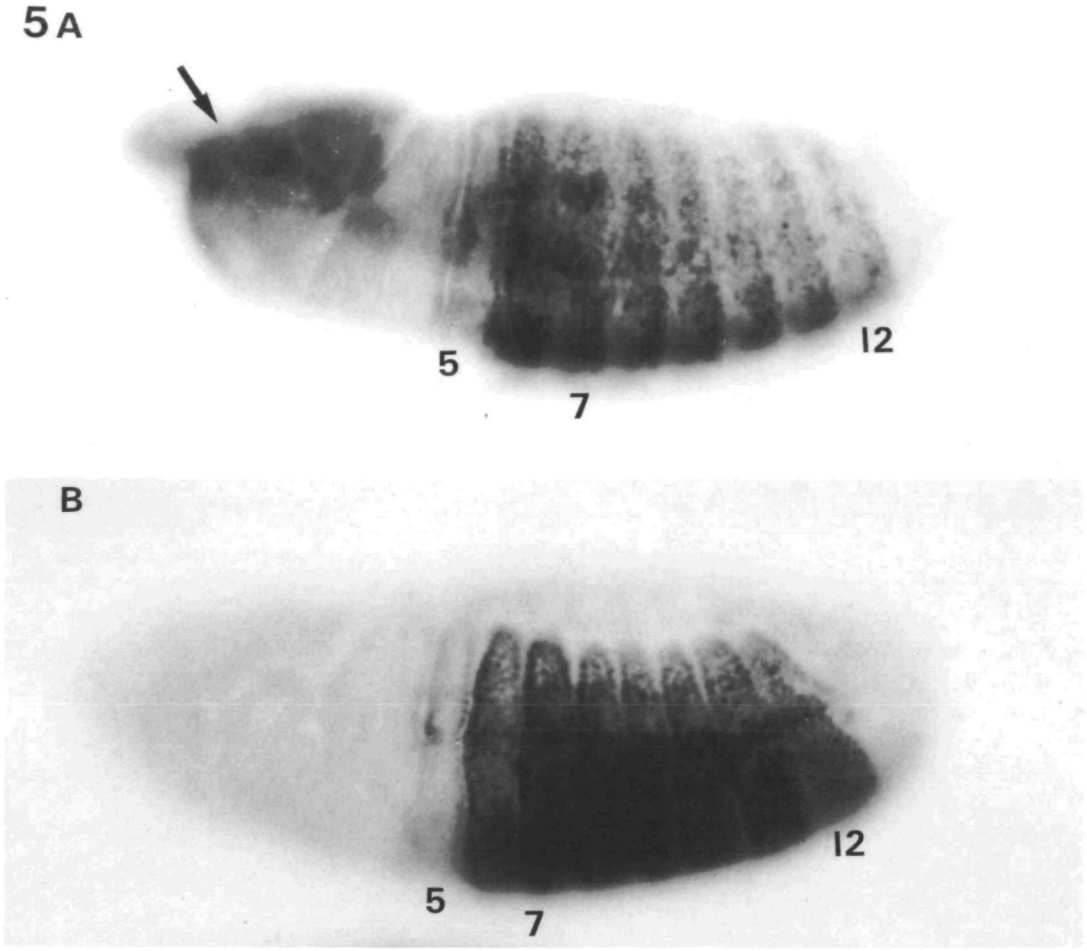


Fig. 5. Expression of *Ubx* in the presence (A) and absence (B) of *abd-A* in embryos doubly stained for *Ubx* and *β-gal* after germ band retraction. The embryo in A is of genotype *abd-A^{M1}/TM3, hb-β-gal* as indicated by the anterior *hb* label (arrow). The one in B lacks the *hb* label and is therefore homozygous for *abd-A^{M1}*. The *Ubx* protein is expressed more uniformly in B and also there is more antigen in the posterior compartments. Some parasegments are indicated.

coincides with that of the wild-type function of the *r* element (DeLorenzi *et al.* 1988; Sánchez-Herrero and Crosby, 1988; Kuziora and McGinnis, 1988).

Discussion

Wild-type expression of abd-A *and its regulation*

Our results show that the embryonic domain of *abd-A* expression extends from parasegments 7 to 13, in good agreement with genetic data based on the phenotypes of *abd-A* mutations (Sánchez-Herrero *et al.* 1985; Busturia *et al.* 1989). The anterior limit of *abd-A* function is strictly parasegmental; double labelling for *abd-A* and *en* indicates a cell-by-cell correspondence in the A1p compartment.

The posterior limit is located within the A8a where some ventral cells express the protein, but this limit does not follow any compartment boundary. The expression of *Ubx* shows a similar phenomenon; only a subset of cells of the A8a compartment contain *Ubx* product (Fig. 1H). The posterior border of *abd-A* expression can be altered by removing gene products of the *Abd-B* gene. Under these conditions, the *abd-A* domain expands to parasegment 15, the last metamere of the body. Thus, in the absence of interactions with other homeotic genes, the genetic machinery of *abd-A* would make the gene active in parasegments 7 to 15. This is unlike *Ubx* which, in the absence of the *trans* interacting genes *abd-A* and *Abd-B* (Struhl and White, 1985; our own results), is only expressed down to parasegment 13, although the amount of product in 13 is higher than in the wild-type.

In the case of *abd-A*, it is remarkable that the expansion of the gene's domain in the absence of *Abd-B* proceeds in a temporal order; by stage 10 (elongated germ band), it abuts the posterior limit of the *m* domain, and by stage 12 (after germ band shortening), it has spread to most or all of the *r* domain (figs 6C and 7). It suggests that there is an early activation of *abd-A* from parasegments 7 to 13, which is down-regulated by high levels of *m* protein in some cells of A8a. A second event of *abd-A* activation would occur later in parasegments 14 and 15, and is normally suppressed by the *r* protein.

Thus for both *abd-A* and *Ubx*, the limits of the

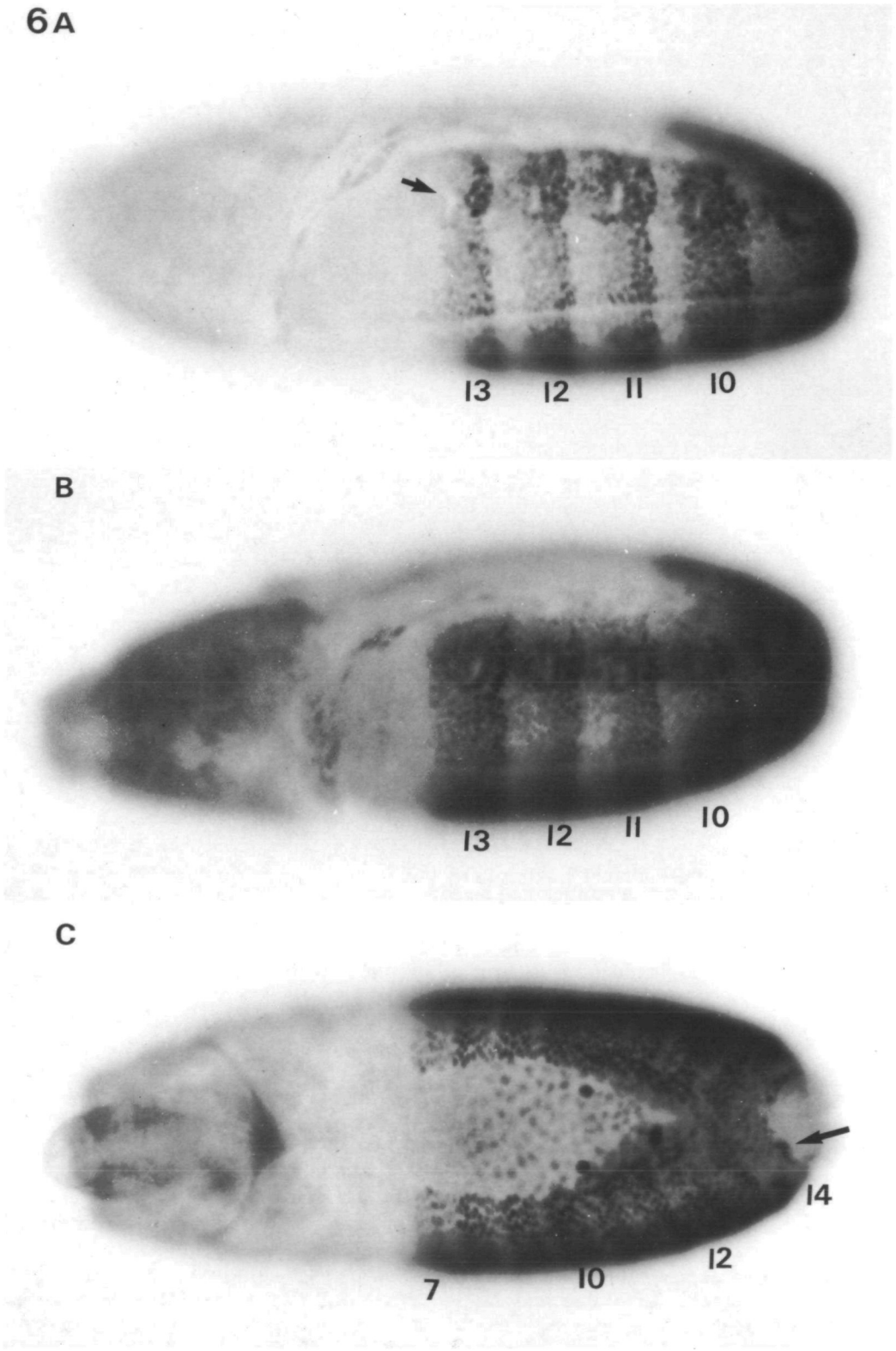


Fig. 6. Expression of *abd-A* in the absence of the *Abd-B* gene. (A) Ventral view of a wild-type embryo at the extended germ band period. Parasegments 10, 11, 12 and 13 are exposed. In 13 there is *abd-A* protein in some cells but not in those posterior to the tracheal pit (arrow). (B) Embryo of the same age as that in A but homozygous for the $Abd\text{-}B^{M1}$ mutation. The *abd-A* label extends to the end of the A8a compartment. Note that the cells posterior to the tracheal pit are marked with the antibody. (C) Dorsal view of a $Abd\text{-}B^{M1}$ homozygous embryo after germ band retraction. The *abd-A* mark (arrow) has now extended to parasegment 14 and some cells of A9p (parasegment 15). Note the label in the amnioserosa cells.

7

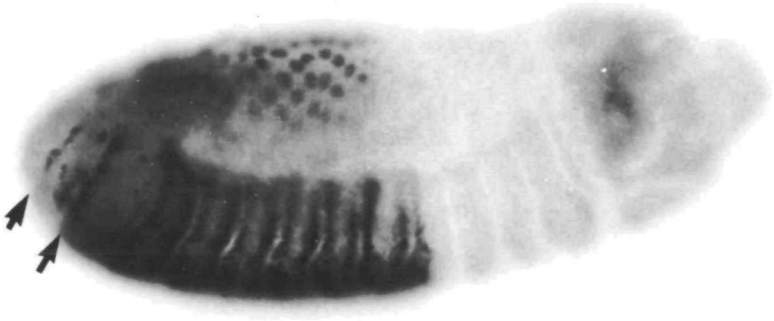


Fig. 7. Effect of the absence of the *r* function of *Abd-B* on the expression of the *abd-A* gene at the shortened germ band period. Embryo of genotype $Abd\text{-}Br^{x231}$ ($m^{+}r^{-}$) showing expression in A8p and A9p (arrows). Notice the low level of expression in A8a compartment where *abd-A* is down-regulated by the *m* product of *Abd-B*.

posterior borders depend on interactions with other homeotic products. Since the results of these interactions may depend, like the case of *abd-A* and *Abd-B*, on threshold levels of product, it is not surprising that they do not follow lineage boundaries. The anterior borders of expression, however, are parasegmental because in those regions, for example, *Ubx* in parasegment 5 or *abd-A* in parasegment 7, these genes are not down-regulated. It will be interesting to see the original domains of activation of the homeotic genes in the absence of *trans* regulatory interactions. If they respond to genes (gap, pair-rule), whose main rule is to establish parasegments, these original domains should be defined by parasegmental boundaries at both ends.

The expression of *abd-A* also shows an internal modulation (Figs 1E,F and 2A,B), repeated in every metamere; there is high level of product in the posterior compartment and in the more anterior cells of the anterior compartment, then it decreases towards the anteroposterior compartment boundary. The establishment of these different levels of product depend on the function of polarity genes. It is already known (Martinez-Arias and White, 1988) that *engrailed* affects the metameric modulation of *Ubx*, although our results suggest that the effect of *en* on *Ubx* may be mediated by *abd-A*. It is not clear whether this modulation in the amount of *abd-A* is important for the metameric identities; at least for *Ubx*, the replacement of the normal metameric pattern of expression by the uniform level induced by an *hsp70-Ubx* gene (Gonzales-Reyes and Morata, 1990) does not produce a detectable alteration in the epidermal pattern.

One novel aspect of the *abd-A* expression is the observation that down-regulation may depend on quantitative levels and not only on the nature of the product; the low levels of *Abd-B* product in segments A5, A6 and A7 (Celniker *et al.* 1989; DeLorenci and Bienz, 1990) does not apparently affect *abd-A* expression, but higher amounts in A8 eliminate *abd-A* activity in most cells. This kind of observation would tend to favour models of interactions based on competition for binding sites (Gibson and Gehring, 1988).

Functional structure of abd-A

Our first conclusion concerning the functional structure of *abd-A* is that all the functions of the gene are executed by the protein encoded by the homeobox-containing transcription unit. The mutations located within it have a very strong phenotype (Busturia *et al.* 1989) and, as we show here, eliminate the *abd-A* protein. Only mutations located in the extreme ends of the transcription unit can still produce some protein, probably because they do not alter the coding region. The mutation *P10* is illustrative in this respect; it is a breakpoint near the 3′ end and *abd-A* protein is synthesized, (Fig. 3B) but some regulatory sequences have probably been eliminated causing an abnormal gradient of expression. The *P10* protein determines a near normal development in some abdominal segments (Morata *et al.* 1983).

The pattern of *abd-A* expression in the *P10* is of interest in connection with the important problem of whether there are parasegment-specific regulatory elements within the gene. All the current models on functional organization of the BX-C (Lewis, 1978; Peifer *et al.* 1987; Casanova *et al.* 1987) suggest that the BX-C is deployed in a metamere-specific manner that is mediated by non-coding, *cis*-acting elements within the gene. Our results with the *P10* deletion are difficult to interpret in the light of this class of model; in *P10*, the whole regulatory region located 3′ to the transcription unit (where the *iab-2* element is located) has been removed, while all the upstream sequences where the *iab-3* and *iab-4* elements reside (Karch *et al.* 1985) are intact. Yet, in *P10* embryos, we still observe some *abd-A* expression in parasegment 7 (Fig. 3B), together with a drastic reduction in parasegments 8, 9 and 10. This indicates, first, that *iab-2* is not the sole element responsible for *abd-A* expression in parasegment 7 and, second, that it is also required in more posterior parasegments where the presence of *iab-3* and *iab-4* is

not sufficient to promote normal levels of expression. Furthermore, most of the mutations in the regulatory regions that we have examined, whether located 5′ or 3′ the transcription unit, give rise to a ectopic expression anterior to parasegment 7. Certainly, the regions of the gene where these mutations map are important for the regulation of the *abd-A* transcription unit, but how they act and which are their physical domains of function still remain obscure.

Role of abd-A *in the acquisition of abdominal patterns*

Previous genetic and developmental analyses (Sánchez-Herrero *et al.* 1985; Busturia *et al.* 1989) have established that the *abd-A* gene has an important role in determining the A2–A8 (or parasegments 7 to 13) identities; a null *abd-A* mutation like $abd\text{-}A^{M1}$ strongly transforms all these segments towards A1 (parasegment 6). The transformed region includes parasegments 10, 11, 12 and 13, even though they belong to the *Abd-B* domain. This observation is consistent with the high level of expression of *abd-A* in part of the *Abd-B* domain (parasegments 10–12 and in part of 13) that we have found, and with published reports (Sánchez-Herrero and Crosby, 1988; Celniker *et al.* 1989; DeLorenzi and Bienz, 1990) indicating a relatively low level of expression of *Abd-B* in the same region. It seems that *abd-A* is the principal homeotic product for the larval abdomen, while *Abd-B* plays a minor role in parasegments 10–12. As suggested by Akam *et al.* 1988, *abd-A* may be the original 'abdominal' gene of the BX-C, while *Abd-B* is mainly responsible for terminal structures (e.g. genitalia, analia) and its influence on abdominal development is a late evolutionary acquisition and mostly limited to adult structures. In fact, the larval patterns of A5, A6 and A7 (parasegments 10–12), which contain *Abd-B* protein, are barely distinguishable from those of A2–A4, which do not contain it. The constancy of the larval patterns in the whole region A2–A7 is also consistent with the similar patterns of *abd-A* expression found in all these embryonic segments.

We thank Jeff Simon for making the *pabexl* clone available to us and Pat Micelli and the Dr Struhl laboratory (Columbia University-HHMI) for help in the antibody work. Thanks also to Gary Struhl for sending the *TM3 hb-β-gal* balancer chromosome and Ernesto Sánchez-Herrero for many discussions and suggestions. This work has been financially supported by the Direccion General de Investigacion Cientifica y Tecnica and by the Fundacion Ramon Areces.

References

Akam, M. E. and Martínez-Arias, A. (1985). The distribution of *Ultrabithorax* transcripts in *Drosophila* embryos. *EMBO J.* **4**, 1689–1700.

Akam, M., Dawson, I. and Tear, G. (1988). Homeotic genes and the control of segment diversity. *Development* **104** (Suppl.) 123–133.

Beachy, P. A., Helfand, S. L. and Hogness, D. S. (1985). Segmental distribution of Bithorax complex proteins during *Drosophila* development. *Nature* **313**, 545–551.

Busturia, A., Casanova, J., Sánchez-Herrero, E., González, R. and Morata, G. (1989). Genetic structure of the *abd-A* gene of *Drosophila*. *Development* **107**, 575–583.

Campos-Ortega, J. A. and Hartenstein, V. (1985). *The Embryonic Development of* Drosophila melanogaster. Berlin: Springer-Verlag.

Casanova, J., Sánchez-Herrero, E., Busturia, A. and Morata, G. (1987). Double and triple mutant combinations of the bithorax complex of *Drosophila*. *EMBO J.* **6**, 3103–3109.

Casanova, J., Sánchez-Herrero, E. and Morata, G. (1985). Prothoracic transformation and functional structure of the *Ultrabithorax* gene of *Drosophila*. *Cell* **42**, 663–669..

Casanova, J., Sánchez-Herrero, E. and Morata, G. (1986). Identification and characterization of a parasegment specific regulatory element of the *Abdominal-B* gene of *Drosophila*. *Cell* **47**, 627–636.

Casanova, J., Sánchez-Herrero, E. and Morata, G. (1988). Developmental analysis of a hybrid gene made of parts of the *Ubx* and *abd-A* genes of *Drosophila*. *EMBO J.* **7**, 1097–1105.

Celniker, S., Keelan, D. J. and Lewis, E. B. (1989). The molecular genetics of the bithorax complex of *Drosophila*: characterization of the products of the *Abdominal-B* domain. *Genes and Dev.* **3**, 1424–1436.

DeLorenzi, M., Ali, N., Saari, G., Henry, C., Wilcox, M. and Bienz, M. (1988). Evidence that the *Abdominal-B r* element function is conferred by a trans-regulatory homeoprotein. *EMBO J.* **7**, 3223–3231.

DeLorenzi, M. and Bienz, M. (1990). Expression of *Abdominal-B* homeoproteins in *Drosophila* embryos. *Development* **108**, 323–329.

Gibson, G. and Gehring, W. J. (1988). Head and thoracic transformations caused by ectopic expression of *Antennapedia* during *Drosophila* development. *Development* **103**, 657–675.

Gonzalez-Reyes, A. and Morata, G. (1990). The developmental effect of overexpressing a *Ubx* product in *Drosophila* embryos depends on its interactions with other homeotic products. *Cell* **61**, 515–523.

Hafen, E., Levine, M. and Gehring, W. J. (1984). Regulation of *Antennapedia* transcript distribution by the bithorax complex in *Drosophila*. *Nature* **307**, 287–289.

Harding, K., Wedeen, C., McGinnis, W. and Levine, M. (1985). Spatially regulated expression of homeotic genes in *Drosophila*. *Science* **229**, 1236–1242.

Jack, T., Regulski, M. and McGinnis, W. (1988). Pair-rule segmentation genes regulate the expression of the homeotic selector gene *Deformed*. *Genes and Dev.* **2**, 635–651.

Karch, F., Weiffenbach, B., Peifer, M., Bender, W., Duncan, I., Celniker, S., Crosby, M. and Lewis, E. B. (1985). The abdominal region of the bithorax complex. *Cell* **43**, 81–96.

Kuziora, M. A. and McGinnis, W. (1988). Different transcripts of the *Drosophila Abd-B* gene correlate with distinct genetic sub-functions. *EMBO J.* **7**, 3233–3244.

Lawrence, P. A. (1982). Cell lineage of the thoracic muscles of *Drosophila*. *Cell* **29**, 493–503.

Lawrence, P. A., Johnston, P., McDonald, P. and Struhl, G. (1987). Borders of parasegments in *Drosophila* embryos are delimited by the *fushi tarazu* and *even-skipped* genes. *Nature* **328**, 440–442.

LeMotte, P., Kuroiwa, A., Fessler, L. I. and Gehring, W. (1989). The homeotic gene *Sex Comb Reduced* of *Drosophila*: gene structure and embryonic expression. *EMBO J.* **8**, 219–227.

Lewis, E. B. (1978). A gene complex controlling segmentation in *Drosophila*. *Nature* **276**, 565–570.

Mahaffey, J. W. and Kaufman, T. C. (1987). The distribution of the *Sex Comb Reduced* gene products during development of *Drosophila melanogaster*. *Genetics* **117**, 51–60.

Mahaffey, J. W. and Kaufman, T. C. (1988). The homeotic genes of the Antennapedia complex and the bithorax complex of *Drosophila*. In *Developmental Genetics of Higher Organisms* (ed. G. M. Malacinsky), pp. 239–260. New York: Macmillan.

Martínez-Arias, A. and Lawrence, P. A. (1985). Parasegments and compartments in the *Drosophila* embryo. *Nature* **313**, 639–642.

Martínez-Arias, A. and White, R. A. H. (1988). *Ultrabithorax* and *engrailed* expression in *Drosophila* embryos mutant for segmentation genes of their pair-rule class. *Development* **102**, 325–338.

MODOLELL, J., BENDER, W. AND MESELSON, M. (1983). *Drosophila melanogaster* mutations suppressible by the *Suppressor of Hairy wing* are insertions of a 7.3 kilobase mobile element. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1678–1682.

MORATA, G., BOTAS, J., KERRIDGE, S. AND STRUHL, G. (1983). Homeotic transformations of the abdominal segments of *Drosophila* caused by breaking or deleting a central portion of the Bithorax complex. *J. Embryol. exp. Morph.* **78**, 319–341.

MORATA, G. AND GARCIA-BELLIDO, A. (1976). Developmental analysis of some mutants of the bithorax system of Drosophila. *Wilhelm Roux Arch. devl Biol.* **179**, 125–143.

MORATA, G. AND LAWRENCE, P. A. (1977). Homeotic genes, compartments and cell determination in *Drosophila. Nature* **265**, 211–216.

PARKHUST, S. M. AND CORCES, V. G. (1986). Interactions among the *gypsy* transposable element and the *yellow* and *suppressor of Hairy wing* loci in *Drosophila melanogaster. Molec. cell. Biol.* **6**, 47–53.

PATEL, N. H., MARTÍN-BLANCO, E., COLEMAN, K. G., POOLE, S. J., ELLIS, M. C., KORNBERG, T. B. AND GOODMAN, C. S. (1989). Expression of *engrailed* proteins in arthropods, annelids and chordates. *Cell* **58**, 955–968.

PEIFER, M. AND BENDER, W. (1986). The anterobithorax and bithorax mutations of the bithorax complex. *EMBO J.* **5**, 2293–2303.

PEIFER, M., KARCH, F. AND BENDER, W. (1987). The bithorax complex: control of segment identity. *Genes and Dev.* **1**, 891–898.

ROWE, A. AND AKAM, M. (1988). The structure and expression of a hybrid homeotic gene. *EMBO J.* **7**, 1107–1114.

SÁNCHEZ-HERRERO, E. AND CROSBY, M. A. (1988). The *Abdominal-B* gene of *Drosophila melanogaster*: overlapping transcrits exhibit two different spatial distributions. *EMBO J.* **7**, 2163–2173.

SÁNCHEZ-HERRERO, E., VERNOS, I., MARCO, R. AND MORATA, G. (1985). Genetic organization of the *Drosophila* Bithorax complex. *Nature* **313**, 108–113.

STRUHL, G. AND WHITE, R. A. H. (1985). Regulation of the *Ultrabithorax* gene of *Drosophila* by other bithorax complex genes. *Cell* **43**, 507–519.

TABOR, S. AND RICHARDSON, C. C. (1985). A bacteriophage T7 RNA polymerase promoter system for controlled exclusive expression of specific genes. *Proc. natn. Acad. Sci. U.S.A.* **82**, 1074–1078.

TREMML, G. AND BIENZ, M. (1989). Homeotic gene expression in the visceral mesoderm of *Drosophila* embryos. *EMBO J.* **8**, 2677–2685.

WHITE, R. A. H. AND LEHMANN, R. (1986). A gap gene, *hunchback*, regulates the spatial expression of *Ultrabithorax*. *Cell* **47**, 311–321.

WHITE, R. A. H. AND WILCOX, M. (1985). Regulation of the distribution of the *Ultrabithorax* proteins in *Drosophila*. *Nature* **318**, 563–566.

WIRZ, J., FESSLER, L. I. AND GEHRING, W. J. (1986). Localization of the *Antennapedia* protein in *Drosophila* embryos and imaginal discs. *EMBO J.* **5**, 3327–3334.

(Accepted 18 September 1990)